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2019-05

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Johannesen , K M , Gardena , E , Encinas , A C , Lehesjoki , A-E , Linnankivi , T , Petersen , M B , Lund , I C B , Blichfeldt , S , Miranda , M J , Pal , D K , Lascelles , K , Procopis , P , Orsini , A , Bonuccelli , A , Giacomini , T , Helbig , I , Fenger , C D , Sisodiya , S M , Hernandez-Hernandez , L , Krithika , S , Rumple , M , Masnada , S , Valente , M , Cereda , C , Giordano , L , Accorsi , P , Burki , S , Mancardi , M , Korff , C , Guerrini , R , von Spiczak , S , Hoffman-Zacharska , D , Mazurczak , T , Coppola , A , Buono , S , Vecchi , M , Hammer , M F , Varesio , C , Veggiotti , P , Lal , D , Bruenger , T , Zara , F , Striano , P , Rohholi , G & Moller , R S 2019 , ' The spectrum of intermediate SCN8A-related epilepsy ' , *Epilepsia* , vol. 60 , no. 5 , pp. 830-844 . <https://doi.org/10.1111/epi.14705>

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<http://hdl.handle.net/10138/312952>

<https://doi.org/10.1111/epi.14705>

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## FULL-LENGTH ORIGINAL RESEARCH

The spectrum of intermediate *SCN8A*-related epilepsy

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## Summary

**Objective:** Pathogenic variants in *SCN8A* have been associated with a wide spectrum of epilepsy phenotypes, ranging from benign familial infantile seizures (BFIS) to epileptic encephalopathies with variable severity. Furthermore, a few patients with intellectual disability (ID) or movement disorders without epilepsy have been reported. The vast majority of the published *SCN8A* patients suffer from severe developmental and epileptic encephalopathy (DEE). In this study, we aimed to provide further insight on the spectrum of milder *SCN8A*-related epilepsies.

**Methods:** A cohort of 1095 patients were screened using a next generation sequencing panel. Further patients were ascertained from a network of epilepsy genetics clinics. Patients with severe DEE and BFIS were excluded from the study.

**Results:** We found 36 probands who presented with an *SCN8A*-related epilepsy and normal intellect (33%) or mild (61%) to moderate ID (6%). All patients presented with epilepsy between age 1.5 months and 7 years (mean = 13.6 months), and 58% of these became seizure-free, two-thirds on monotherapy. Neurological disturbances included ataxia (28%) and hypotonia (19%) as the most prominent features. Interictal

electroencephalogram was normal in 41%. Several recurrent variants were observed, including Ile763Val, Val891Met, Gly1475Arg, Gly1483Lys, Phe1588Leu, Arg1617Gln, Ala1650Val/Thr, Arg1872Gln, and Asn1877Ser.

**Significance:** With this study, we explore the electroclinical features of an intermediate *SCN8A*-related epilepsy with mild cognitive impairment, which is for the majority a treatable epilepsy.

#### KEYWORDS

epilepsy, epilepsy genetics, intellectual disability, *SCN8A*, voltage-gated sodium channels

## 1 | INTRODUCTION

*SCN8A* encodes the voltage-gated sodium channel Na<sub>v</sub>1.6, which is primarily expressed in excitatory neurons with high concentrations at the axon initial segment and the node of Ranvier. Pathogenic variants in *SCN8A* are associated with a spectrum of epilepsy phenotypes, ranging from rare families with benign familial infantile seizures (BFIS) to severe early onset developmental and epileptic encephalopathies (DEE; EIEE13, Online Mendelian Inheritance in Man database #614558). Since the first case report published by Veeramah and colleagues in 2012,<sup>1</sup> several reports have confirmed the role of *SCN8A*, primarily in patients with DEE.<sup>2–9</sup> The majority of patients with *SCN8A* DEE described so far have a severe phenotype characterized by early seizure onset, difficult to treat seizures, severe intellectual disability (ID), motor disorders, and a relatively high mortality.<sup>2–10</sup>

Functional studies of selected variants causing DEE have revealed gain of function (GOF) as the main pathogenic mechanism.<sup>1,6,11,12</sup> This GOF comes from hyperactivity of the ion channel, due to elevated persistent sodium currents, hyperpolarizing shifts in the voltage dependence of activation, or impaired channel current inactivation.<sup>12,13</sup> This mechanism is the opposite of the one that has been demonstrated in Dravet syndrome, which is characterized by loss-of-function (LOF) variants in *SCN1A*.<sup>14</sup> *SCN1A* encodes the voltage-gated sodium channel Na<sub>v</sub>1.1, which is primarily expressed in inhibitory interneurons. Variants in *SCN2A*, encoding a third voltage-gated sodium channel in the human central nervous system, can lead to both GOF and LOF, complicating things even more.<sup>15</sup> Awareness of these important differences in pathophysiology is necessary, as it might have therapeutic implications.<sup>16</sup>

We have recently detected a recurrent *SCN8A* variant, Glu1483Lys, in a very mild familial epilepsy phenotype.<sup>17</sup> We identified three unrelated families with a total of 16 family members who presented with BFIS and normal cognition. Five family members developed paroxysmal kinesigenic dyskinesia.<sup>17</sup> Recently, Han et al confirmed BFIS as part of the

#### Key Points

- The phenotypic spectrum of *SCN8A* is wide, from BFIS to DEE
- The intermediate phenotype is characterized by a treatable epilepsy and mild cognitive delay
- Prognostic markers remain elusive

phenotypic spectrum in *SCN8A*-related epilepsies.<sup>18</sup> In addition to these two extreme phenotypes, there are an increasing number of patients with a milder form of DEE. In this study, we describe the spectrum of the electroclinical features of this intermediate *SCN8A* epilepsy phenotype, aiming to provide a clearer picture for clinicians, genetic counselors, and affected families.

## 2 | MATERIALS AND METHODS

We systematically screened all exons and exon-intron boundaries of *SCN8A* in a cohort of 1095 unselected patients with various forms of epilepsy using different next generation sequencing (NGS) panels.<sup>19</sup> The panels included from 45 to 500+ genes related to epilepsy, intellectual disability, or autism. Variants were assumed to be pathogenic if they arose de novo, or were inherited from an affected parent or affected/unaffected mosaic parent, and if they were nonsynonymous, splice-site altering, or frameshift causing, and not present in controls in the gnomAD browser (see Web Resources). Sanger sequencing confirmed variants and segregation. Furthermore, detected variants were tested (PolyPhen-2, SIFT, and MutationTaster) for predicted pathogenicity. American College of Medical Genetics and Genomics (ACMG) and Missense badness, PolyPhen-2, and Constraint (MPC) scores are noted in Table 1. All variants, except for one, were either pathogenic or likely pathogenic according to the ACMG criteria.<sup>20</sup>

Four different metrics were used for in silico variant pathogenicity prediction:

**TABLE 1** Clinical characteristics of the probands

Proband #	Age at inclusion	Variant, inheritance, location	Pathogenicity per ACMG guidelines, MPC	Family history	Age at SZ onset
1	2 y 8 mo	c.-8A>G de novo 5'UTR	Pathogenic	None	11 mo
2	9 y	c.411C>G p.Ile137Met maternal transmembrane domain D1S1	Likely pathogenic (PM1, PM2, PP1, PP2, PP3) MPC = 1.66	Mother, at 10-11 y: episodes of loss of balance, lower limb hyposthenia and falls, gait disturbance and hand tremor	2 y 2 mo
3	9 y 4 mo	c.1122C>G p.Asn374Lys de novo pore-forming domain	Likely pathogenic (PS2, PP2, PP3) MPC = 1.83	Maternal cousin with SZ	7 y
4		c.1630_1631del p.Asn544 fs*39 maternal cytoplasmic domain D1/D2	Pathogenic (PVS1, PM2, PP1)	See Figure 1	2 y 9 mo
5	13 y	2287A>G p.Ile763Val de novo transmembrane domain D2S1	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC = 1.51	Cousin with febrile SZ	7 wk
6	20 y	c.2287A>G p.Ile763Val de novo transmembrane domain D2S1	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC = 1.51	None	3 mo
7	11 y	c.2671G>A p.Val891Met maternal transmembrane domain D2S5	Pathogenic (PS1, PM1, PM2, PP1, PP2) MPC = 3.14	See Figure 1	3 y
8	10 y	c.2806G>A p.Glu936Lys nonmaternal pore-forming domain	Likely pathogenic (PM1, PM2, PP1, PP2) MPC = 3.33	Father with ID and ADHD	5 y
9	9 y 9 mo	c.3601G>A p.Glu1201Lys de novo transmembrane domain D3S1	Likely pathogenic (PS2, PM1, PM2, PP2) MPC = 2.37	None	8 mo
10	2 y	c.3722A>G p.Tyr1241Cys pending transmembrane domain D3S2	VUS (PM1, PP2) MPC = 2.48	None	8 mo
11	2 y 1 mo	c.3953A>G p.Asn1318Ser de novo cytoplasmic linker D3S4/D3S5	Likely pathogenic (PS2, PM2, PP2) MPC = 2.04	None	3 mo
12	2 y 3 mo	c.3956C>A p.Ala1319Asp de novo cytoplasmic linker D3S4/D3S5	Likely pathogenic (PS2, PM2, PP2) MPC = 2.69	None	2 mo
13	7 y 11 mo	c.3967G>A p.Ala1323Thr, de novo cytoplasmic domain D3S4/D3S5	Likely pathogenic (PS2, PM2, PP2) MPC = 2.22	None	5 mo
14		c.4391T>C p.Ile1464Thr paternal inactivation gate	Likely pathogenic (PM1, PM2, PP1, PP2) MPC = 2.65	See Figure 1	7 mo
15	10 y 6 m	c.4423G>A p.Gly1475Arg, inactivation gate	Likely pathogenic (PS1, PM2, PP1, PP2) MPC = 1.54	See Figure 1	9 mo
16	9 mo	c.4423G>A p.Gly1475Arg de novo, inactivation gate	Pathogenic (PS1, PS2, PP2) MPC = 1.54	None	4 mo
17	9 y 9 mo	c.4423G>A p.Gly1475Arg de novo blood mosaicism 10% inactivation gate	Pathogenic (PS1, PS2, PP2) MPC = 1.54	None	11 mo

SZ types	Interictal EEG/ictal EEG	Cognition before SZ onset/cognition after SZ onset	Treatment response	Successful monotherapy	Other features
GTC, AA	EDs on the P-temporal regions bilaterally, with rare diffuse spreading	N/MID	SZ reduction: VPA 50%		Hypotonia, uncoordinated movements, gait disturbance
Staring/hypotonia to GTC, A, F (visual)	Temporal-O spikes/SW (left side predominance)	MID/MID	SZ reduction: LTG, VPA, ZNS; no effect: LEV, RFM, TPM		Hypotonia, hypothyroidism enuresis, sleep disorders
Nocturnal frontal, T, GTC, SE	Multifocal SW, paroxysmal beta activity that lasts 1-2 min	N/MID	SZ-free: PHT, VPA; no effect: OXC, LEV, ZNS, CLB		Gait disturbance
Ab	Slow wave and SW discharges	N/MID	SZ-free: VPA + ESM + AMD; SZ reduction: VPA + ESM		
GTC	NA	NA/MID	SZ-free: CLB, ZNS, PHT, LTG, CBZ; adverse effects: LEV (behavior), CLZ, and LZP (sleepiness)		Hypotonia, ataxia, chronic constipation, premature adrenarche, periodic leg movement
S, F	EDs, both hemispheres; ictal: right F-temporal onset, then rhythmic slow activity propagating into the right parasagittal regions	NA/MID	No effect: VPA, CBZ, LTG, CLB, acetazolamide		Ataxia
GTC, AA	CP and midline spikes	N/MID	SZ-free: CBZ	CBZ	ADHD
F, AA, FS	SW left F, C, right P	NA/MID	SZ-free: LEV; adverse effects: TPM	LEV	Autistic features, behavioral problems
M	Initially normal (1 y-3 y 4 mo), then BG slowing (7-10 y); postictal: abundant beta, diffusely slow BG	DD/MID	SZ reduction: VPA, LEV, CLB; no effect: STP; SZ aggravation: OXC		Hypotonia, ataxia, paroxysmal dystonia
F, A	Focal posterior bilateral EDs	NA/MID	SZ-free: VGB	VGB	Paroxysmal dyskinesia
F, GTC	Normal; ictal: EDs on the P regions bilaterally	N/N	SZ reduction: CBZ, PER; no effect: PB, OXC, CLB, ZNS; adverse effects: TPM (hyperthermia)		Ataxia
C, T, GTC	Normal; ictal EEG: parasagittal EDs, F rhythmic delta	N/MID	SZ reduction: PHT; no effect: VPA, PB		Ataxia, tremor, language delay
FS, GTC	Normal	N/N	SZ-free: VPA	VPA	
A, GTC, SE	Diffuse abnormal	NA/MID	SZ reduction: TPM, VPA		
F to GTC, AA	Slowing over the temporal region (3 y), then normal (9 y)	NA/MID	SZ-free: LTG; SZ reduction: OXC, CBZ; no effect: LEV	LTG	Hypotonia, ataxia, autistic features
F, T	Ictal: slowing and EDs on both hemispheres, mainly temporal regions	NA/MID	SZ-free: PHT; no effect: LEV	PHT	Hypotonia, ataxia
GTC, F	Normal	N/N	SZ-free: CBZ; SZ reduction: VPA	CBZ	

(Continues)

TABLE 1 (Continued)

Proband #	Age at inclusion	Variant, inheritance, location	Pathogenicity per ACMG guidelines, MPC	Family history	Age at SZ onset
18	8 y	c.4447 G>A, p.Glu1483Lys de novo inactivation gate	Pathogenic (PS1, PS2, PS3, PM2, PP2) MPC = 2.13	None	11 mo
19	2 y	c.4585A>G p.Met1529Val de novo transmembrane D4S1	Likely pathogenic (PS2, PM1, PM2, PP2) MPC = 1.2	None	4 mo
20	11 y	c.4764C>G p.Phe1588Leu de novo transmembrane D4S3	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC = 2.4	None	3.5 mo
21	13 y	c.4764C>A p.Phe1588Leu de novo transmembrane D4S3	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC = 2.4	None	5 mo
22	2 y 8 mo	c.4840A>G p.Thr1614Ala de novo extracellular domain D4S3/D4S4	Likely pathogenic (PS2, PM2, PP2) MPC = 2.17	None	4.5 mo
23	7 y 5 mo	c.4850G>C p.Arg1617Gln de novo transmembrane D4S4	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC = 2.4	None	5 mo
24	9 y	c.4850G>A p.Arg1617Gln de novo transmembrane D4S4	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC = 2.4	Paternal first cousin with microcephaly	5 mo
25	24 y	c.4850G>A p.Arg1617Gln de novo transmembrane D4S4	Pathogenic (PS1, PS2, PM1, PM2, PP2) MPC = 2.4	None	NA
26	7 y	c.4949 C>T p.Ala1650Val de novo cytoplasmic D4S4/D4S5	Pathogenic (PS1, PS2, PM2, PP2) MPC = 2.26	None	11 mo
27	9 y	c.4961T>A p.Ile1654Asn unknown transmembrane domain D4S5	Likely pathogenic (PM1, PM2, PP2) MPC = 2.92	None	2 y 8 mo
28	4 y	c.5273T>C p.Val1758Ala de novo transmembrane D4S6	Likely pathogenic (PS2, PM1, PM2, PP2) MPC = 2.46	None	2 y
29	9 y	c.5311G>A p.Val1771Ile de novo C-terminal	Likely pathogenic (PS2, PM2, PP2) MPC = 2.02	None	6 mo
30	6 y	c.5458C>T p.Arg1820* de novo C-terminal	Pathogenic (PVS1, PS1, PM2, PP2)	None	3-4 mo
31	15 y	c.5497G>C p.Asp1833His unknown C-terminal	Likely pathogenic (PM1, PM2, PP2) MPC = 2.44	None	6 mo
32	3 y	c.5597G>A, p.Arg1866Gln, de novo C-terminal	Likely pathogenic (PS2, PM2, PP2) MPC = 2.39	None	9 mo
33		c.5615G>A p.Arg1872Gln paternal (mosaic) cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP1, PP2) MPC = 2.39	See Figure 1	6 wk
34	14 y	c.5630A>G, p.Asn1877Ser, pending cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP2) MPC = 2.04	None	5 mo

SZ types	Interictal EEG/ictal EEG	Cognition before SZ onset/cognition after SZ onset	Treatment response	Successful monotherapy	Other features
F, GTC	Normal background, small spikes/theta pointu in the central regions	N/N	Sz-free: VPA, CBZ, currently no AED		Clumsy, shy, speech delay
GTC	Right posterior slow activity during sleep; ictal: generalized spike discharges	N/N	No effect: LEV, PB, VPA		
GTC, AA, T, F	Normal	DD/MID	SZ-free: CBZ	CBZ	Autism, language delay
F, GTC	NA	N/MID	SZ reduction: PB, CBZ		Obesity
AA, GTC, M, A	Generalized SW	N/MID	SZ reduction: VPA, OXC		
GTC	Normal	DD/MD	SZ-free: LTG	LTG	Autism
GTC, M, A	NA	DD/MID			Language delay, paroxysmal dyskinesia
GTC, F	NA	MID/MID	SZ-free: CBZ, VPA		Extrapyramidal signs
M, A, T, S, AA	Generalized EDs	NA/MID	SZ-free: CLB, RUF, KD; adverse effects: LEV (increased frequency), ZNS (new SZ type), VPA (neurodevelopmental regression)		Hypotonia, supraventricular tachycardia
Ab	Generalized 3-Hz SW; spikes occipital right and left	NA/MID	No effect: VPA		Ataxia
Ab, FS	Mild BG slowing, irregular generalized EDs, OIRDA; ictal: irregularly generalized, 3-Hz SW followed by rhythmic diffuse delta activity (clinically: arrest of ongoing activity)	N/N	SZ-free: ETX; no effect: LEV	ETX	
GTC, T	Normal	N/N	SZ reduction: CBZ		
M in hands and fingers	Trains of SW, right CP; irregular delta activity on the left side	NA/MID	SZ reduction: LTG		Ataxia, language delay
T	EDs over the posterior lobe	N/N	SZ-free: PB; no effect: LEV	PB	Reflux
F, A, AA	EDs, mid CP	N/mild learning disability	SZ-free: CBD; no effect: CBZ, CLB, LEV, TPM, VGB, VPA, ST; adverse effects: CBZ (drowsiness and severe cognitive disturbance)	CBD	
F, clusters of GTC, SE	Normal	N/N	SZ-free: CBZ + LEV		Learning difficulties
F clustering	Normal	N/N	SZ-free: LTG	LTG	Language delay

(Continues)



TABLE 1 (Continued)

Proband #	Age at inclusion	Variant, inheritance, location	Pathogenicity per ACMG guidelines, MPC	Family history	Age at SZ onset
35	4 y 4 mo	c.5630A>G Asn1877Ser paternal cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP1, PP2) MPC = 2.04	See Figure 1	6 mo
36	4 y 6 mo	c.5630A>G p.Asn1877Ser unknown cytoplasmic c-terminal	Likely pathogenic (PS1, PM2, PP2) MPC = 2.04	None	7 mo

A, atonic; AA, atypical absences; Ab, absences; ACMG, American College of Medical Genetics and Genomics; ADHD, attention-deficit/hyperactivity disorder; AED, antiepileptic drug; AMD, amantadine; BG, background; C, clonic; CBD, cannabidiol; CBZ, carbamazepine; CLB, clobazam; CLZ, clonazepam; CP, cerebral palsy; DD, developmental delay; ED, epileptiform discharges; EEG, electroencephalogram; ESM, ethosuximide; ETX, ethosuximide; F, focal; FC, focal clonic; FS, febrile seizures; GTC, generalized tonic-clonic; ID, intellectual disability; KD, ketogenic diet; LEV, levetiracetam; LTG, lamotrigine; LZP, lorazepam; M, myoclonic; MD, moderate intellectual disability; MID, mild intellectual disability; MPC, missense badness, PolyPhen-2, and constraint; N, normal; NA, not available; O, occipital; OIRDA, occipital intermittent rhythmic delta activity; OXC, oxcarbazepine; P, parietal; PB, phenobarbital; PER, perampanel; PHT, phenytoin; PP1, pathogenicity possible 1; PP2, pathogenicity possible 2; PP3, pathogenicity possible 2; PS1, pathogenicity strong 1; PS2, pathogenicity strong 2; PS3, pathogenicity strong 3; PVS1, pathogenicity very strong 1; RFM, rufinamide; RUF, rufinamide; S, spasms; SE, status epilepticus; STP, stiripentol; SW, spike and wave complexes; SZ, seizure(s); T, tonic; TPM, topiramate; UTR, untranslated region; VGB, vigabatrin; VPA, valproic acid; VUS, variant of unknown significance; ZNS, zonisamide.

1. The MPC score, which demonstrates a 5.8-fold increased variant enrichment in cases compared to individuals from the general population for MPC scores > 2.<sup>21</sup>
2. The paralogue conservation score (parazscore), which quantifies amino-acid position conservation across human proteins of the same gene family. A significant enrichment of disease-associated missense variants was observed at paralogue-conserved sites.<sup>22</sup>
3. The Grantham score, which accesses the effect of the amino acid substitution based on the properties of the amino acid exchange. It ranks amino acid substitutions from similar amino acids substitutions (0) to substitutions that differ in their chemical properties.<sup>23</sup>
4. The allele frequency analysis based on the alleleFrequencyApp (see Web Resources), which calculates a maximum credible number of possible pathogenic alleles observed in gnomAD. The allele count is estimated based on the disease prevalence, the allelic and genetic heterogeneity, and the variant penetrance. For *SCN8A*, we specified the disease prevalence as one in 300, the allelic heterogeneity as 0.01, the genetic heterogeneity as 0.1, and the penetrance as 50%, resulting in a maximum number of a single allele in gnomAD. We compared the corresponding value with the allele frequency of variants present in gnomAD.

*SCN8A*-positive patients underwent a detailed clinical evaluation, and patients with severe DEE and BFIS were excluded from the study. The criteria for severe DEE were defined as severe, pharmacoresistant epilepsy, and developmental impairment, as previously reported.<sup>24</sup> BFIS is a self-limiting epilepsy syndrome characterized by afebrile seizures, typically in clusters, with onset between 4 and 8 months. Neurological examination, psychomotor development, and the interictal electroencephalogram (EEG) are normal, and the children become seizure-free within the first

years of life and sustain seizure freedom without the aid of antiepileptic drugs (AEDs).<sup>25</sup>

Additional probands were collected from an international network of epilepsy genetics clinics. Data on clinical phenotype, genetics, neuroimaging, and EEG were requested for all patients (and if possible, relatives) who were included in the study. Seizures were classified according to the International League Against Epilepsy.<sup>26</sup> All probands, and in the case of minors, legal parents, provided written informed consent. The study was approved by the local ethical committees.

Sodium channel blockers (SCBs) were defined as AEDs that target sodium channels and included carbamazepine (CBZ), oxcarbazepine, lamotrigine (LTG), and phenytoin (PHT).

## 2.1 | Data sharing

Anonymized data will be shared by request from any qualified investigator.

## 3 | RESULTS

By the use of targeted NGS screening of *SCN8A* in a cohort of 1095 patients, we identified 12 (1.2%) probands with a predicted pathogenic variant in *SCN8A*. Six of the patients fulfilled the criteria for a severe DEE and thus were excluded from this study. Of the 1095 patients screened, approximately 326 fulfilled a DEE diagnosis (this might be an underestimate, as referrals to our center are often lacking clinical information). In addition to the remaining six patients, we ascertained 30 additional probands/families with predicted pathogenic *SCN8A* variants through collaborating diagnostic and research laboratories. The Asn1877Ser variant was seen in three controls in gnomAD, but as the

SZ types	Interictal EEG/ictal EEG	Cognition before SZ onset/cognition after SZ onset	Treatment response	Successful monotherapy	Other features
	Normal	N/MID	SZ-free: LTG, VPA		
GTC, T, AA	Sporadic EDs, FC bilateral > right	N/MID	SZ reduction: VPA, PB, TPM		Ataxia, clumsiness

phenotype resembles a benign familial epilepsy, with several family members affected, and was seen in three probands in this study, it was included as being pathogenic. All variants, except #10 p.Tyr1241Cys, were classified as pathogenic or likely pathogenic according to the ACMG criteria.<sup>20</sup> Not all missense variants in *SCN8A* are pathogenic, and at 384 amino acid positions variants have been reported in the general population.<sup>27</sup>

Distinguishing disease-causing variants from benign variants is still a challenge in clinical genetics. Rarity of an allele is widely recognized as a necessary (although not sufficient) criterion for variant pathogenicity (ACMG guidelines, but the key question “how common is too common?” remains poorly answered for many diseases).<sup>20</sup> Estimating the genetic and allelic heterogeneity offers an opportunity to identify variant cutoff frequency filters. Genetic heterogeneity is the maximum proportion of disease that is attributable to variation in a single gene, and allelic heterogeneity is the maximum proportion of variation within a gene that is attributable to a single allele.

The patient variant 10 (c.3722A>G, p.Tyr1241Cys, NM\_014191.3) was found once in the gnomAD<sup>27</sup> and discoverEHR<sup>28</sup> database. The presence of a variant in databases does not represent a pathogenicity exclusion criterion based on our allele frequency analysis for mild forms of epilepsy. The amino acid residue position is relatively conserved across voltage-gated sodium channels (positive parazscore = 0.23<sup>22</sup>). In addition, the MPC score (MPC = 2.48) supports variant pathogenicity and the Grantham score of 194 indicates a likely pathogenic variant.

One of the probands (#30) has previously been mentioned briefly,<sup>29</sup> but was included in this study because additional data had been collected.

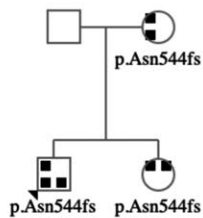
In total, 36 probands/families were included. The detected *SCN8A* variants were mainly missense variants (33/36)

scattered throughout the gene. In addition, two truncating variants (one frameshift [#4] and one stop [#30]), and one variant causing a nucleotide change eight base pairs upstream of the start codon (#1) were detected. Fourteen variants were located in the transmembrane domains and two in the pore-forming domain; of the cytoplasmic variants, we found one in the D1/D2 domain. The variant Asn544 fs\* was a frameshift variant with a clinical picture of absence epilepsy, inherited from an affected parent, and classified as pathogenic according to the ACMG classification guidelines.

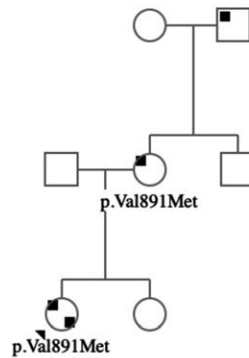
The variants either occurred de novo or segregated within the family in a dominant fashion (#2, #4, #7, #14, #15, #33, and #34; see Figure 1 for selected pedigrees). In the family of Proband #33, the variant was inherited from an affected mosaic parent. Mosaicism was also suspected in #15, but has not yet been confirmed. All variants were located at highly conserved residues and were predicted to be possibly damaging according to computational prediction software (see Materials and Methods and Web Resources). Mining the available literature and databases, nine variants were found to be recurrent either within this study or overall; Ile763Val,<sup>30</sup> Val891Met,<sup>31</sup> Gly1475Arg,<sup>10,32</sup> Gly1483Lys,<sup>17</sup> Phe1588Leu, Arg1617Gln,<sup>5,7,8,33,34</sup> Ala1650Val<sup>5,7,35</sup> (different amino acid substitution, see Discussion section), Arg1872Gln<sup>7,12,36</sup> and Asn1877Ser,<sup>30,35,37</sup> with five of them (amino acid positions 763, 1475, 1617, 1650, and 1872) seen in severe DEE phenotypes as well.

Clinical data on all 36 probands and families are presented in Table 1. Age at inclusion varied from 9 months to 35 years, with a mean of 7.9 years. Seizure onset was between 6 weeks and 7 years, with a mean of 13.6 months (SD = ±17 months). Seizure semiology was very diverse, and included generalized tonic-clonic (n = 21), focal (n = 14), tonic (n = 8), myoclonic (n = 6), and atonic seizures (n = 8), as well as epileptic spasms (n = 2) and atypical/typical absences (n =

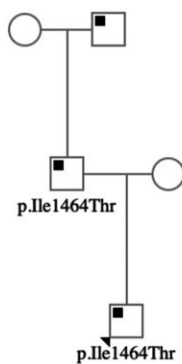
Family of proband #4



Family of proband #7



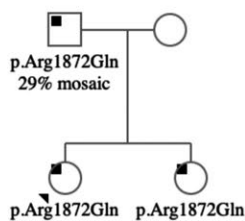
Family of proband #14



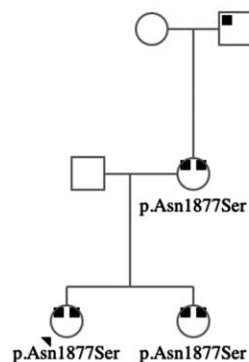
Family of proband #15



Family of proband #33



Family of proband #35

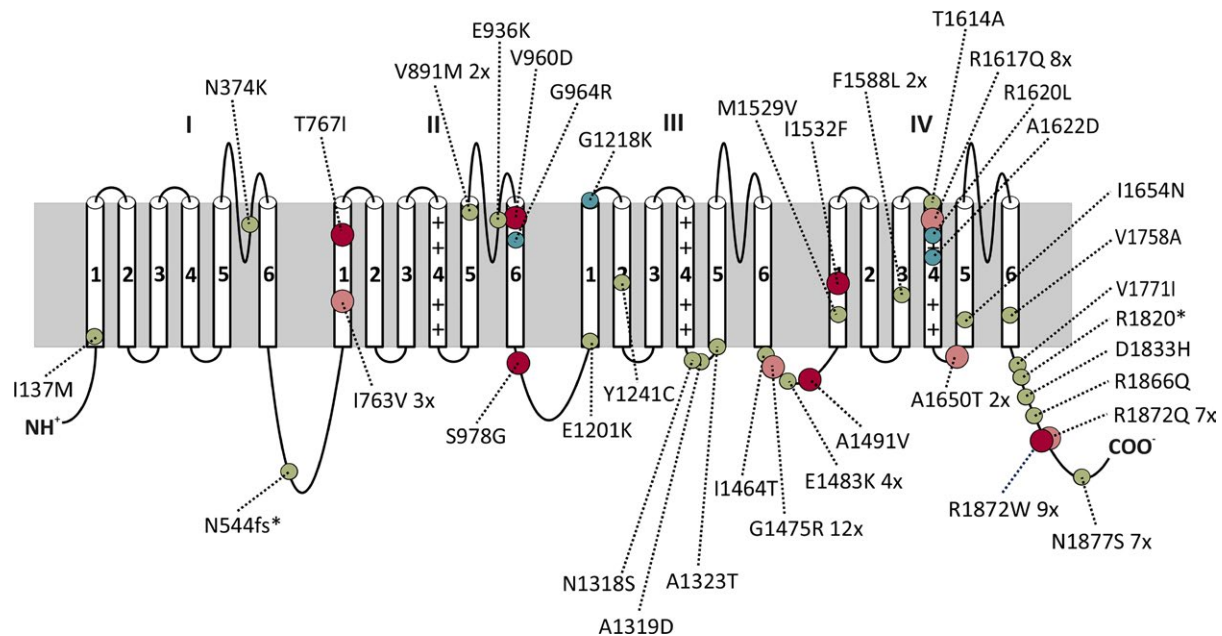


**FIGURE 1** Pedigrees of *SCN8A*-related epilepsies showing segregation of the variant with the phenotype. ID, intellectual disability

12). Seizure severity did not progress over time, and seizure triggers were not found. Three probands experienced convulsive status epilepticus (#3, #14, #33).

The interictal EEG was available in 33 of 36 patients and showed focal epileptiform abnormalities in 15 patients (45%), predominant in the posterior quadrants in (10 patients) or in the central/centroparietal/frontocentral regions (five patients),

with or without bilateral spreading. Four patients (12%) had only generalized abnormalities (#6, #14, #22, #26). In 12 patients (39%), the interictal EEG was normal (#9, #11, #12, #13, #16, #17, #20, #23, #29, #34, #35) or normalized at follow-up (#15). Ictal EEG was available in four patients, showing a focal discharge in two (#6, #11) and generalized spike or irregular spike and waves discharges in the other two (#19, #28).



**FIGURE 2** A partial display of published variants, with the recurrent developmental and epileptic encephalopathy (DEE) and known loss-of-function variants, as well as those identified in this study. Blue indicates intellectual disability or ataxia without epilepsy.<sup>44,47</sup> Green indicates intermediate epilepsy (this study). Red indicates DEE variants (previously published<sup>4,7,24</sup>). Pink indicates recurrent variants: DEE and intermediate epilepsy

Cognitively, these patients fare well. Before seizure onset, 26 of 36 (72%) had normal cognitive development, two of 36 (6%) had a mild ID, one of 36 had moderate ID (3%), and four of 36 (11%) had developmental delay, not classified due to the young age of the patient. At follow-up, a deterioration from normal intellect/developmental delay to mild ID or from mild ID/developmental delay to moderate ID was seen in seven (19%) patients, whereas 81% did not experience deterioration after seizure onset. At the time of follow-up, 22 of 36 (61%) had mild ID and two of 36 (6%) had moderate ID.

Additional features included ataxia (10/36), hypotonia (7/36), language delay (5/36), autism/autistic features (4/36), movement disorders (3/36) including paroxysmal dyskinesia (3/36), gait disturbances (2/36), sleep disturbances (1/36), learning difficulties (2/36), and attention-deficit/hyperactivity disorder (1/36). See Table 1 for details.

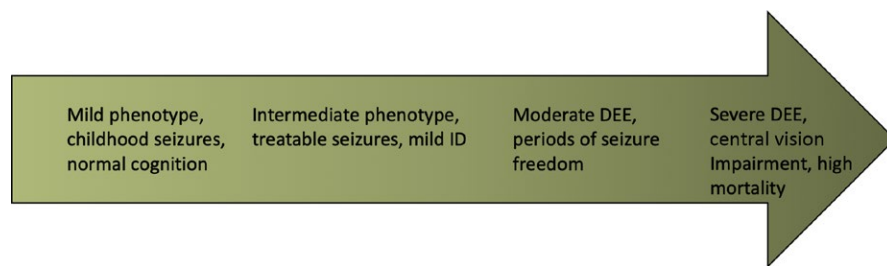
All probands were evaluated for their treatment response. Twenty-one of 36 (58%) probands became seizure-free. Monotherapy was sufficient in 12 of 21 (57%) probands, and included LTG ( $n = 3$ ), CBZ ( $n = 2$ ), PHT ( $n = 1$ ), valproate ( $n = 2$ ), vigabatrin ( $n = 1$ ), ethosuximide ( $n = 1$ ), phenobarbital ( $n = 1$ ), and levetiracetam ( $n = 1$ ). In total, 13 of 36 (36%) probands became seizure-free with therapy that included an SCB either in monotherapy or in combination with other AEDs. One became seizure-free with a small dose of cannabidiol. However, seven of 36 (19%) probands became seizure-free without the use of SCBs. Seizure offset was only available for six patients,

and ranged between 4 and 10 years; mean age at seizure offset was 7.7 years.

Six probands had affected family members, segregating with the variants. For Proband #5, a similar phenotype with absences and learning/language difficulties was seen in the sister, as well as the mother. For Proband #7, the mother also had unspecified epilepsy and carried the variant; there was also an affected maternal grandfather, who did not have genetic analysis done. Likewise, Proband #14 had an affected father, who carried the variant, and an affected paternal grandfather, who was not tested genetically. Proband #15 had a sister with a similar epilepsy phenotype; the variant was suspected to have been inherited from a mosaic parent, but this was not confirmed at the time of preparation of this paper. Proband #33 had an affected sister with a similar phenotype, and genetic investigations showed that the variant was inherited from an affected mosaic (28%) father. Proband #34 had an affected sister and mother, in whom the variant segregated.

## 4 | DISCUSSION

Pathogenic variants in *SCN8A* have so far been described in patients with different epilepsy phenotypes, including rare families with BFIS and in >100 patients with mild to severe DEE. In this study, we describe the electroclinical phenotype of 36 patients with intermediate epilepsies due to pathogenic variants in *SCN8A*. Patients with



**FIGURE 3** The spectrum of *SCN8A*-related epilepsy. DEE, developmental and epileptic encephalopathy; ID, intellectual disability; SUDEP, sudden unexpected death in epilepsy

*SCN8A*-related severe DEE<sup>21</sup> and BFIS<sup>18</sup> were excluded from the study.

The variant at position c.-8A>G was assumed to be likely pathogenic because it occurred de novo and the clinical features of the patient resembled the phenotype seen in other *SCN8A* patients. The variant is located outside of the Kozak consensus sequence, but may lead to increased RNA stability or translational initiation or result in altered splicing pattern. These theories can only be confirmed by functional testing of the variant, which unfortunately was not possible in this study. Until then, the variant may need to be classified as a variant of unknown significance.

All 36 patients presented with seizures in early childhood (mean = 13.6 months). Before seizure onset, 72% probands had normal cognitive development. More than half of the probands became seizure-free, 57% of these with monotherapy. Compared to the BFIS families, described by Gardella et al,<sup>17</sup> Anand et al,<sup>37</sup> and Han et al,<sup>18</sup> the majority of the probands in this cohort have cognitive impairment, with 6% suffering from moderate ID and 61% from mild ID. Furthermore, only 58% became seizure-free compared to almost 100% of the BFIS patients; seizure freedom is exceptional in the severe DEE phenotype. The patients herein described also appeared to have additional neurological disturbances, including primarily ataxia (in 28%) and hypotonia (in 19%). In the severe DEE cohort, the incidence of ataxia is around 11%, compared to the 28% of this cohort. However, it is important to note that many of the patients suffering from severe *SCN8A* DEE are unable to walk autonomously. Furthermore, the patients in this intermediate cohort do not suffer from the spasticity and paraplegia or the extrapyramidal/cerebellar symptoms that up to 50% of the severe DEE patients do.<sup>24</sup> A few patients in this cohort (8%) had dyskinesia, which is also seen in both severe DEEs and BFIS families. Growth impairment (microcephaly or reduced growth), observed in severe DEE, was not seen in this cohort. Other prominent phenotypic features included language delay/difficulties in 14% and movement disorders not further specified in 8%.

In mouse models, it has been shown that *SCN8A* is widely expressed, both in the motor neurons of the brainstem and in many types of neurons in the cerebellum, where functional deficits in Purkinje cells have been found,<sup>38–40</sup> confirming the importance of *SCN8A* in motor function.

This could explain the involvement of the motor system, and why cerebellar atrophy and ataxia,<sup>41</sup> associated with intellectual impairment, appeared to be major features in subjects harboring *SCN8A* variants. The cerebellum does, however, also play an important role in language and grammar processing, verbal working memory, and speech motor planning,<sup>42</sup> and a large proportion of *SCN8A* patients, including those reported in the present cohort, show an impairment of these functions as well.

When comparing the present cohort to the severe *SCN8A* DEE population, in which patients have earlier seizure onset, with more pronounced cognitive deficits as well as refractory epilepsy, we tried to identify possible predictive factors that in newly diagnosed patients could help to detect those with a milder course as compared to those with a more severe evolution. First, within our cohort, the majority of probands have normal development prior to seizure onset (and 33% of them continue to develop normally) and maintain a normal EEG (41%). This is often not the case in probands who develop severe DEE, in which the majority usually are cognitively delayed from birth,<sup>43</sup> or will present with cognitive difficulties early on and show changes in their EEGs. However, it is not an absolute feature, as we have observed several children with an extremely severe condition at follow-up, despite early normal development. Second, seizure onset occurred at a mean age of 13.6 months, compared to a mean age of 4 months in the DEE group.<sup>43</sup> It is worth bearing in mind that the age deviation in this cohort is quite large (SD = ±17 months), and thus, these numbers should be interpreted carefully when counseling a family. Last, seizure freedom was obtained in 58% of the patients in this cohort, and it was achieved rapidly and with monotherapy in two-thirds. In contrast, only about 5% of patients in the severe DEE cohort achieve seizure freedom with monotherapy. This is important, albeit also a prerequisite in this study, where the more severe epilepsies were excluded.

Previously, it has been hypothesized that seizures in patients with *SCN8A* variants should respond to SCBs.<sup>16,17</sup> A beneficial effect of SCBs was observed in 36% of the patients reported in this study, either as monotherapy or in combination with other AEDs, and supportive of this, previous studies have shown a GOF of the Arg1617Gln and Arg1872Leu<sup>12</sup> variants. However, 19% became seizure-free without the use of SCBs, suggesting that seizure freedom should not be



attributed solely to the use of SCBs, but also should be considered a phenotypic trait.

Interestingly, a partial benefit of LTG was also observed in the proband with a stop variant (#30). We can speculate that this unexpected finding may depend on genetic modifiers and differences in genetic background, or may be because as the stop-codon is located at the c-terminal part of the protein, the transcript does not undergo nonsense-mediated decay.

Furthermore, we found three variants, one eight base pairs upstream of the start codon (#1), one frameshift variant (#4), and one stop variant (#30), all suspected to be LOF and all associated with an epilepsy phenotype. Previously, it has been hypothesized that LOF variants would not cause epilepsy,<sup>44</sup> but rather present with ID with or without motor function abnormalities, such as ataxia.<sup>2,34,41</sup> However, Blanchard et al also describe a patient with epilepsy and ID, carrying an LOF variant,<sup>11</sup> and two existing *Scn8a* knockout mice models support the notion of epilepsy also being a feature of LOF of NaV1.6.<sup>45,46</sup> Other ion channel genes including *SCN2A* have been shown to have a similar clinical picture, where both GOF and LOF variants may cause epilepsy. The underlying functional causes are yet to be fully elucidated.

Variants were found throughout the gene (see Figure 2), including the transmembrane segments, cytoplasmic loops, and inactivation gate, and thus, location in the gene is likely not predictable of functional or clinical effects of the variant; however, the figure does display that the majority of the variants are found in domains three and four, the inactivation gate and the c-terminal, which may help guide variant interpretation.

We identified several recurrent variants and observed a wide range of phenotypic variability for variant carriers. Ile763Val, Gly1475Arg, Arg1617Gln, Ala1650Val, and Arg1872Gln were all seen in this study, as well as in patients with DEE. Ile763Val has previously been described in a patient with intractable epilepsy and moderate ID,<sup>30</sup> whereas we found it in two patients with focal epilepsy and mild ID (#5 and #6).

The Gly1475Arg variant is even more diverse, and it has previously been identified in several patients, including a child with severe DEE, who died from probable sudden unexpected death in epilepsy.<sup>10,32</sup> In this cohort, we found it in two patients with mild ID, and epilepsy was controlled by SCBs. Both patients did suffer from hypotonia and ataxia.

Arg1617Gln has been seen in several DEE patients previously, including a girl with severe DEE who died after terminal progression of her disease,<sup>2,17</sup> whereas we found it in two patients with mild ID and treatable epilepsy (#24, #25). However, they did display additional neurological disturbances (language delay, dyskinesia) and their seizure onset was earlier (4 months), compared to the rest of this cohort. We also found the Arg1617Gln variant in one patient (#21), who displayed moderate ID as well as autism. Ala1650Val variant has not been described before (#26), but has been

seen several times with a threonine (Ala1650Thr) substitution in patients with severe DEE.<sup>5,7,35</sup> Arg1872Gln has also been seen several times in patients with severe DEE<sup>7,36</sup>; however, we found it in a sib pair, with normal intellect and focal epilepsy (#34). The Gly1483Lys variant has previously been described in BFIS<sup>17</sup>; the patient in this study (#18) carrying this variant has a very mild phenotype, with only speech delay, sporadic seizures, and discrete focal EEG abnormalities. Why some variants show phenotypic heterogeneity and other variants do not remains elusive. Of course, differences in the amino acid substitution might explain some of the difference, but this is true for just a few of the variants. In other cases, genetic modifiers and differences in the genetic background could underlie these observations. Further studies are needed to investigate this further.

In conclusion, in this study we provide further insight into the phenotypic spectrum of *SCN8A* epilepsy by focusing on an intermediate phenotype characterized by treatable epilepsy with a later age of onset, mildly impaired cognitive development, and variable but in general mild neurological disturbances. A positive response to epilepsy treatment, especially with SCBs, was observed. Even if a wide range of phenotypes related to *SCN8A* variants can be expected (see illustration in Figure 3), these findings highlight the presence of an increasing number of *SCN8A* patients with a phenotype of moderate severity.

The partial overlapping of genetic and early clinical features in *SCN8A*-related epilepsies makes it difficult to provide proper counseling in these children so far. Further investigations are warranted to clarify this issue, as well as to explore possible prognostic factors.

## ACKNOWLEDGMENT

We thank the patients and families for participating in our study.

## CONFLICT OF INTEREST

None of the other authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## WEB RESOURCES

ExAC browser: <http://exac.broadinstitute.org>  
 gnomAD database: <http://gnomad.broadinstitute.org/>  
 SIFT: <http://provean.jcvi.org/index.php>  
 PolyPhen-2: <http://genetics.bwh.harvard.edu/pph2/>  
 MutationTaster: <http://www.mutationtaster.org>  
 Allele frequency app: <https://www.cardiodb.org/allele-frequencyapp>

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**How to cite this article:** Johannesen KM, Gardella E, Encinas AC, et al. The spectrum of intermediate SCN8A-related epilepsy. *Epilepsia.* 2019;00:1–15.  
<https://doi.org/10.1111/epi.14705>